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Original Article

Hypothalamic–Pituitary–Gonadal Activity in Paradoxical and Psychophysiological Insomnia

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Abstract

Background: Although insomnia is a sex-dimorphic disorder, there is limited knowledge about the association between sex hormones and insomnia. In the present study, the level of hypothalamus–pituitary–gonadal (HPG) axis activity was investigated in patients with insomnia by measuring serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), 17α-Hydroxyprogesterone, testosterone, progesterone, estradiol, dehydroepiandrosterone sulfate, and sex hormone-binding globulin. **Methods:** Numbers of 19 patients; including 13 females (68.40%) with paradox insomnia (32–53 years; 43.20 ± 6.40) and 17 patients; including 8 females (47.05%) with psychophysiological insomnia (14–62 years; 38.40 ± 16.30) were recruited. Seventeen aged-matched normal sleeper consisted of 13 males (26–59 years; 40.70 ± 10) consisted of 13 males (76.50%) were also recruited as control group. Insomnia was diagnosed by a sleep clinician according to the International Classification of Sleep Disorders-Second Edition criteria and an overnight polysomnography (PSG). A volume of 5 ml of venous blood samples were collected, prepared, and stored at 8 AM under standard condition. Serum levels of hormones were measured using enzyme-linked immunosorbent assay kits. Data were analyzed by Chi-square and ANCOVA. The associations between PSG and biochemical parameters were evaluated using multiple linear regression analysis. **Results:** There were no significant differences in all biochemical analyses between two insomnia subgroups (paradoxical and psychophysiological insomnia) and normal sleepers. Testosterone was positively related to maximum pulse transit time (PTT). Moreover, both LH and FSH were positively related to wake index and diastolic blood pressure. **Conclusion:** Although there were no significant differences in all HPG's hormones between groups, both LH and FSH were associated with wake index and diastolic blood pressure. Moreover, testosterone was positively related to PTT.

Keywords: *Electroencephalographic signal processing, hypothalamic–Pituitary–Gonadal axis, insomnia, polysomnography*

Introduction

Sleep is an essential neurophysiologic phenomenon that plays a critical role in body homeostasis.[1] Poor sleep quality and sleep deficiencies could affect the endocrine systems in a way that may disturb the individual health.[2-4] Insomnia as a more prevalent sleep disorder affect 10% of adults.[5] Patients with insomnia have difficulties in initiating and maintaining sleep. They also may awake early in the morning but inable to return to sleep. These problems should occur at least three times per week for insomnia disgnosis. This neurophysiologic phenomenon produces clinically significant impairment in social and occupational functioning.[6] Despite the significant effect on the quality of life, its biological base has not been fully understood. Both men and women follow two different patterns of sleep habits.[7] It has been well established that insomnia is a sex-dimorphic and female-biased disorder. Previous studies indicated that females are more susceptible to insomnia rather than males.[8-11] Despite this gender dependency of insomnia, our understanding about the relation between sex hormones and sleep quality is insufficient.

The essential effects of sex steroids on brain structure and function have been documented. Sex steroids contribute to development and function of the neural system.[12,13] Moreover, steroids can regulate neurotransmission and electrophysiological properties of neurons by bind to synaptic membrane receptors temporarily.[12] Different sex steroid receptors in many regions of the brain

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mediate the role of sex hormones on brain functions.[14] Higher susceptibility of women to insomnia has been related to hormonal fluctuation resulted from the menstrual cycle and menopausal status.[15] The direct relationship between sex steroids and sleep quality has been observed in hormone therapy studies,[16] menopausal status,[17] and menstrual cycle in women.[18] In addition, the effect of testosterone on obstructive sleep apnea (OSA) in men confirm the direct relationship between sex steroids and sleep quality.[19] Accordingly, lower sleep quality is related to lower level of testosterone, and this relation is stronger in older individuals. Previous studies revealed that total sleep deprivation significantly reduces the levels of testosterone and luteinizing hormone (LH). In addition, testosterone and estradiol have been related to objective and subjective sleep quality in women aged 48–59 years.[20] Also, testosterone level is positively related to sleep quality and efficiency[19] and negatively related to sleep disorders such as OSA.[21] It has been reported that estrogen and progesterone could influence sleep characteristics. Previous studies indicated that the activity of hypothalamus–pituitary–gonadal (HPG) axis disturbed by sleep deprivation.[22,23] Moreover, there is also a positive association between dehydroepiandrosterone sulfate (DHEA-S) and subjective sleep quality.[24] However, objective sleep monitoring has not been used so far to investigate the association between the activity of HPG axis and sleep characteristics.[25] In addition, insomnia has been defined as a diverse phenomenon. Different subtypes of insomnia have been introduced according to subjective and objective sleep characteristics.[26] Considering this diversity for investigating the endocrinological consequences of sleep disorders may reveal more precise finding about the interaction between sleep and endocrinology. In general, "insomnia" as a whole term was used in HPG data reports; however, there are rare data on paradoxical insomnia, and this subtype of insomnia was not usually considered as a separate diagnosis in biochemical studies. Psychophysiological and paradoxical insomnia are two main subtypes of the disorder which has not been pathophysiologically well understood.[27] Paradoxical insomnia or sleep state misperception in literature is characterized by reports of little or no sleep over long periods of time despite near-normal objective sleep architecture. Paradoxical insomnia has been introduced as subjective insomnia, pseudo-insomnia, subjective sleepiness, and sleep hypochondriasis in the literature in accordance with our perception about its clinical and neurophysiological characteristics.[28]

Sleep state misperception is a diagnostic term adopted in ICSD instead of subjective insomnia complaint without objective findings. In ICSD-2, the paradoxical insomnia was used and considered as a subtype of the disorder.[26] In addition, psychophysiological, paradoxical, and idiopathic insomnia were introduced for primary insomnia by the American Academy of Sleep.[29] In the ICSD-3, the term sleep state misperception was replaced with paradoxical insomnia.[30] In the present study, we considered the term paradoxical insomnia to refer to sleep state misperception condition, and ICSD-2 criteria were considered for diagnosing and classification of participants.

Objective short sleep duration has been proposed as a reliable marker of the biological severity of the insomnia disorder. Insomnia with objective short sleep duration is the most severe subtype of the disorder. This type of insomnia activate both sympathetic system and hypothalamus–pituitary–adrenal [HPA] axis and elevate the risk of hypertension, diabetes, neurocognitive impairment, and mortality. In contrast, paradoxical insomnia defined as insomnia with normal objective sleep duration is related to cognitive-emotional problems and cortical arousal but not with activation of both sympathetic system and hypothalamus–pituitary–adrenal [HPA] axis as limbs of the stress system.[31] Pulse transit time (PTT) as a blood pressure marker related to autonomic nervous system (ANS) activity is a variable measured by polysomnography (PSG) procedure that may reveal association between HPG, stress system, and sleep disturbances. PTT is a time needed for receiving a pulse wave from heart to the finger as a photoplethysmogram (PPG) measurement site.[32]

Assuming that HPG activity may be differently affected by paradoxical and psychophysiological insomnia, we aimed to investigate the final and intermediate products of HPG system among a group of psychophysiological and paradox insomnia patients in comparison with the normal sleeper. Moreover, the associations between HPG activity and sleep architectures as well as autonomic activity were also investigated.

Materials and Methods

Participants

Participants for insomnia groups (including psychophysiological and paradoxical insomnia) were selected from patients referred to Sleep Disorders Research Center (SDRC) of Kermanshah University of Medical Sciences (KUMS) due to insomnia complaint. Thirty-six patients with insomnia complaint; including 21 females (58.30%) and 15 males (41.70%) aged 14–62 years (41 ± 12.20 years) were agreed to participate in this study. All patients were diagnosed by sleep clinician according to the International Classification of Sleep Disorders-Second Edition (ICSD-2)[26] criteria at SDRC. Seventeen aged-matched volunteers with normal sleep consisted of 4 females (23.50%) and 13 males (76.50%) aged 26–59 years (40.70 ± 10 years) were recruited from Kermanshah province for the study as control group. All women participants were in the follicular phase of the ovarian cycle, and they recruited for sleep analysis and blood sampling after ends of menses days. In addition, they did not have any menstrual disorders. We just recruited females <55 years for the exclusion of postmenopausal

women. Furthermore, any postmenopausal symptoms were considered as exclusion criteria for females.

A psychiatrist experienced in sleep medicine and PSG interviewed participants in both patient and control groups. Psychiatric disorders, chronic medical conditions, substance abuse, and chronic neurological, cardiovascular, and respiratory disorders were considered as exclusion criteria. Medications with a narcotic effect were stopped for at least 2 weeks. Other sleep disorders including circadian sleep-wake disorders, hypersomnias, parasomnias, and restless legs syndrome identified by physician's examinations, and sleep-disordered breathing and periodic limb movement disorder identified by PSG were also excluded.

The Ethics Committee of KUMS approved the study. All procedures performed in the study were in accordance with the ethical standards of the Ethics Committee of KUMS and with the 1964 Helsinki declaration and its later amendments. Detailed written informed consent was obtained from all participants.

Insomnia diagnosis

The diagnosis of insomnia was based on clinical interview by a sleep clinician according to ICSD-2.[26] First, the clinician interviewed patients with insomnia complaint referred to SDRC. In addition, all normal sleep volunteers were interviewed according to ICSD-2 criteria for ruling out any sleep disorders. Only participants with insomnia in patient group and volunteers without any sleep disorder were selected for the second step of the study.

At the next step, all selected participants were invited to a whole night PSG procedure. Participants completed the demographic questioner and Persian version of Pittsburgh sleep quality index (PSQI).[33] Height and weight were measured by digital scales and presented as cm and kg respectively to calculate the body mass index (BMI). Then, the sleep recording was started by PSG. The diagnosis of insomnia in patient group and ruling out any sleep disorders in the control were confirmed by PSG analysis.

Subjective sleep investigation

We used PSQI to evaluate subjective sleep characteristics.[34] It is a self-reported questionnaire for the measurement of sleep quality over the past month. The questionnaire consists of 19 items, forming seven components that produce one global score. Components include sleep quality, sleep onset latency (SOL), total sleep time (TST), sleep efficiency (SE), sleep disturbances, the use of sleeping medication, and the daytime dysfunction. TST, SE, and SOL were considered for analysis in the present study.

Polysomnography procedure

Participants were invited to sleep laboratory of SDRC one day before the experiment. They advised not to have coffee, tea, heavy diet, and cigarette as well as snooze and sleep during the day of the experiment. They arrived to the laboratory at 9 pm. The height and weight were measured by an experienced technician, and the participants completed the questioners. Then the PSG procedure was explained to the subject. PSG room was standardized for any noise and visual stimulus based on international standards.[35]

All participants underwent an overnight PSG (SOMNOscreen plus®, Somnomedics, Germany) to dignose and determine the severity and type of the insomnia disorder. PSG were performed based on the subject's usual sleeping habits, and a minimum of 7 h of sleep was recorded for each patient.

The guideline of the American Academy of Sleep Medicine was considered for PSG recording. Twenty-four recording electrodes were prepared, including 14 electroencephalogram channels (C4A1, C3A2, F3, F4, C3, C4, A1, A2, O1, O2, F3A2, F4A1, O1A2, O2A1), 6 electrooculogram channels (EOG1, EOG2, EOG1A1, EOG2A1, EOG1A2, EOG2A2), 3 electromyogram channels (EMG, EMG1, EMG2), and an ECG channel. All recordings were sampled at the rate of 256 Hz. Electroencephalogram (EEG) was recorded using Ag/AgCl electrodes and 10–20 system of electrode placement. In addition, flow (by oronasal thermistor and nasal air pressure transducer), thoracic and abdominal respiratory effort (induction plethysmography), oximetry, and body position were recorded. Respiration was monitored by nasal pressure transducers and oronasal thermocouples. Continuous pulse oximetry and thoracoabdominal movements were also measured.

The following PSG findings introduced as normal sleep pattern and considered as inclusion criteria for normal group: (a) SOL of <15 min; (b) SE of more than 85%, and (c) TST of >7 h. Paradoxical insomnia was determined by the perception of short sleep duration and insomnia complaint despite near-normal objective sleep patterns.[36] Criteria for diagnose of paradoxical insomnia were: (i) An objective TST and SE more than 6 h and 30 min and 85% respectively on overnight PSG; ii) Significant discrepancies between objective (PSG) and subjective (self-report) sleep measures (i.e., a difference of 60 min or more for TST, or a difference of at least 15% for SE). Paradoxical insomnia was dignosed if chronic pattern of self-reported short sleep duration had been reported for at least 6 months, indicated that the symtoms were not associated with substance abuse and other sleep disorders.[29] Finally, patients with insomnia complaints that failed to reach the above-mentioned criteria considered as psychophysiological insomnia. According to PSG results, four males in control group were excluded due to OSA symptoms.

Among PSG data pool, TST, SE, SOL, number of awakening, percent of different sleep stages, sleep arousal index, blood oxygen saturation level (SpO_2), number of

awakening, diastolic blood pressure, wake after sleep onset (WASO), and PTT were extracted and considered for further analysis.

SOL is a time interval between reclining in bed and the onset of sleep.[37] Sleep arousal is a brief awakening (at least 3 s) without return to consciousness characterized by increased EEG, muscle and heart activity.[38] Sleep arousal index in both rapid eye movement (REM) and non-REM (nREM) stage defines as a sudden interruption in the brain sleep pattern of EEG activity. Hence, the arousal index is the average number of arousals per hour.[39] Number of awakening is a number of transitions from sleep to wakefulness, lasting more than 15 s. Wake index identified as the number of awakening events per hour of sleep.[40,41] SpO_2 is peripheral capillary oxygen saturation. Using pulse oximetry, the percentage of oxygenated hemoglobin is measured compared to the total amount of hemoglobin in the blood.[42] WASO is a total amount of time awake after excluding SOL.[43] PTT is a time needed for receiving a pulse wave from heart to the finger as a PPG measurement site. It has been considered as a blood pressure marker and related to the (ANS) activity.[32] Diastolic blood pressure considered as a bottom number indicates the pressure in the arteries when the heart rests between beats.[44]

Biochemical analysis

Next morning after PSG recordings, 5 ml of venous blood samples were collected at 8 AM under standard condition. After preparation, the serum was stored at −20°C for the following analyses according to the standard protocols.

Serum levels of DHEA-S (Code 5175-300; Monobind Inc.), sex hormone-binding globulin (SHBG) (Code 9125-300; Monobind Inc.), testosterone (Code 3725-300; Monobind Inc.), estradiol (Code 4925-300; Monobind Inc.), progesterone (Code 4825-300; Monobind Inc.), 17α-Hydroxyprogesterone (17-OH) (Code 5225-300; Monobind Inc.), follicle stimulating hormone (FSH) (Code 425-300; Monobind Inc.), and LH (Code 625-300; Monobind Inc.,) were measured by using enzyme-linked immunosorbent assay kits. The level of absorbance was read using Awareness Technology STAT FAX 2100 Microplate Reader (Awareness Technology, USA). Hormones levels were calibrated according to the standard calibration curve. Data were presented as pg/mL for estradiol, nmol/L for SHBG, mlU/mL for FSH and LH, and ng/mL for testosterone, progesterone, DHEA, and 17-OH.

Statistical analysis

Participants were divided into paradoxical and psychophysiological insomnia as well as normal sleeper groups. Data were analyzed between groups by Chi-square and ANCOVA. *Post hoc* Tukey multiple comparisons were used to detect the significant differences between groups. Related hormones data and sleep characteristics were compared between three groups based on age, gender, and BMI set as covariates. Based on the important effect of sex on sex-steroids, all biochemical parameters were compared between groups in both male and female subgroups separately. Multiple linear regression analysis was used to evaluate the associations between PSG sleep characteristics and biochemical parameters considering age, gender, and BMI as covariates. All model assumptions were evaluated by residual analysis. Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, USA) version 16.0 was used for the statistical analysis.

Results

Demographic findings

We recruited 53 individuals with mean age of 40.92 ± 11.50 years. They included numbers of 17 (31.2%) normal sleeper and 36 insomniac patients. According to clinical interview, subjective sleep data collected by PSQI and PSG investigation, 19 participants; including 13 females (68.40%) and 6 males (31.60%) were diagnosed as patients with paradoxical insomnia (32–53 years; 43.2 ± 6.4) and 17 participants; including 8 females (47.05%) and 9 males (52.95%) were identified as psychophysiological insomnia patients (14–62 years old; 38.40 ± 16.30).

Demographic characteristics of three studied groups were demonstrated in Table 1. As indicated in the table, three groups were age and BMI-matched. Since there were significantly higher numbers of females in paradoxical and psychophysiological insomnia, sex was considered as a covariant in analysis.

Polysomnography and Pittsburgh sleep quality index findings

We considered subjective TST, SE, SOL and PSQI total score for subjective sleep characteristics. In addition, objective TST, SE, SOL, percentage of sleep stages, REM arousality, nREM arousality, wake index, and WASO obtained from PSG were considered for analysis. We considered sex, age, and BMI as covariate in all analyses. According to ANCOVA test results, subjective TST, SE, SOL, and total PSQI scores were significantly different between three groups ($P < 0.01$).

Tukey *post hoc* analysis indicated significantly lower TST in paradoxical insomnia group compared to normal sleepers ($P < 0.01$) and psychophysiological insomnia group ($P < 0.01$). SOL in paradoxical insomnia group was significantly higher than normal sleepers and psychophysiological insomnia ($P < 0.01$). In addition, SE of paradoxical insomnia group was lower than both normal sleepers ($P < 0.01$) and psychophysiological insomnia ($P < 0.01$) [Table 2].

According to ANCOVA test results, objective TST, SE, wake index, and WASO was significantly different between three-studied groups ($P < 0.01$). In comparison to subjective PSQI results, Tukey *post hoc* analysis test revealed

significantly lower objective TST among psychophysiological insomnia group compared to normal sleepers ($P = 0.01$), but the difference between psychophysiological and paradoxical insomnia groups was not significant ($P = 0.07$). In addition, psychophysiological insomnia group indicate significantly lower objective SE compared to normal sleepers ($P < 0.01$) and paradox insomnia ($P = 0.04$). Objective sleep latency was not significantly different between three groups ($P = 0.07$). PSG wake index is significantly higher among psychophysiological insomniac group compared to normal sleepers ($P = 0.01$) and paradoxical insomniac group ($P = 0.04$). In addition, WASO is significantly higher among psychophysiological insomniac group compared to normal group ($P < 0.01$) and paradoxical insomniac group ($P = 0.02$) [Table 2].

Biochemical findings

All biochemical analyses were compared using ANCOVA test, after adjustment of sex, BMI, and age. Furthermore, the biochemical parameters were compared between groups in two sex subgroup separately. According to the test results, there were no significant differences between normal sleepers and two subtypes of insomnia totally and in male and female subgroups [Table 3].

Associations between PSG sleep structures and biochemical parameters were analyzed by linear regression model adjusted by sex, age, and BMI. According to the results, diastolic blood pressure significantly predict FSH (Coefficient = 1.29; $P < 0.01$), LH (Coefficient = 0.34; $P = 0.04$), and SHBG (Coefficient = 1.37; $P = 0.03$). In addition, wake index was significantly associated with FSH (Coefficient = 1.30; $P < 0.01$) and LH (Coefficient = 0.54; $P < 0.01$). Testosterone was predicted significantly by PTT (Coefficient = 0.31; $P < 0.01$) [Table 4].

Discussion

Sleep characteristics and sleep disorders have been considered as sexual dimorphic phenomena.[7,8,10,45] This

Table 1: Demographic characteristics of the studied groups

	Normal (*n*=17), *n* (%)	Paradoxical insomnia (*n*=19), *n* (%)	Psycophysiological insomnia (*n*=17), *n* (%)	*P*
Age†	40.76 (10.10)[a]	43.26 (6.45)[a]	38.47 (16.36)[a]	0.46[c]
Sex				0.02[b]
Female	4 (23.50)	13 (68.40)	8 (47.05)	
Male	13 (76.50)	6 (31.60)	9 (52.95)	
BMI	26.57 (3.82)[a]	26.55 (3.80)[a]	26.97 (6.54)[a]	0.96[c]

†Mean (SD). Data compared with [c]ANCOVA and [b]Chi-square test. Means with the same superscript letters within a row were not significantly different ($P>0.05$). SD – Standard deviation

Table 2: Sleep structures among normal sleepers and insomnia patients

	Normal (*n*=17)	Paradoxical insomnia (*n*=19)	Psychophysiological insomnia (*n*=17)	*P*
PSQI findings				
Total scores	8.21 (4.73)[a]	15.16 (3.62)[b]	12.00 (5.94)[a,b]	<0.01
TST† (h)	5.57 (1.93)[a]	2.25 (2.46)[b]	4.60 (2.73)[a]	<0.01
SOL (h)	0.79 (0.57)[a]	3.01 (1.24)[b]	1.39 (1.32)[a]	<0.01
SE	74.78 (25.96)[a]	28.88 (20.49)[b]	58.28 (32.66)[a]	<0.01
PSG findings				
TST (h)	7.11 (0.44)[a]	6.82 (0.69)[a]	6.02 (1.58)[b]	<0.01
SE	92.79 (4.32)[a]	88.04 (7.28)[a]	77.25 (19.94)[b]	<0.01
SOL (h)	7.84 (6.60)[a]	9.13 (6.58)[a]	15.30 (26.40)[a]	0.07
Stage 1 (%)	32.44 (16.39)[a]	32.56 (16.54)[a]	32.60 (13.51)[a]	0.41
Stage 2 (%)	21.67 (9.19)[a]	20.21 (10.27)[a]	16.28 (9.33)[a]	0.32
Stage 3 (%)	30.65 (18.39)[a]	23.94 (15.12)[a]	19.17 (16.82)[a]	0.15
REM (%)	8.01 (8.30)[a]	11.32 (8.86)[a]	9.13 (9.79)[a]	0.81
REM arousality	20.50 (11.05)[a]	23.86 (12.62)[a]	24.31 (18.28)[a]	0.93
nREM arousality	24.76 (9.02)[a]	24.74 (6.26)[a]	22.99 (5.43)[a]	0.69
Average pulse transit time	305.93 (10.78)[a]	299.79 (17.91)[a]	308.24 (14.72)[a]	0.23
Wake index	2.02 (0.68)[a]	3.54 (1.92)[a]	8.92 (10.81)[b]	<0.01
WASO	14.53 (4.68)[a]	23.36 (11.94)[a]	40.58 (28.32)[b]	<0.01

†Mean (SD). Statistical analysis for the equality of the mean values among three groups was evaluated using ANCOVA adjusted by sex, age, and BMI ($P<0.05$). Means with the same superscript letters within a row were not significantly different ($P>0.05$). TST – Total sleep time; SE – Sleep efficiency; SOL – Sleep onset latency; REM – Rapid eye movement stage; nREM – non-rapid eye movement stage; WASO – Wake after sleep onset; SD – Standard deviation; BMI – Body mass index; PSG – Polysomnography; PSQI – Pittsburgh sleep quality index

findings originated mainly from studies investigated hormone therapy,[16] menopausal status,[17] and menstrual cycle[18] in women. However, data about the direct effect of sleep architectures on hormonal changes especially in the context of insomnia subtype is restricted. In the present study, we aimed to investigate HPG axis activity in insomnia patients in comparison with normal sleepers by measuring serum levels of DHEA, SHBG, testosterone, estradiol, progesterone, 17-OH, FSH, LH. Moreover, we also investigated the associations between objective sleep measurements obtained by PSG and serum level of biochemical parameters in a multivariate regression model.

The subjective and objective sleep architectures in our study indicated sleep state misperception condition in paradoxical insomnia similar to previous findings.[46,47] Objective TST and SE were significantly lower in psychophysiological insomnia compared to both normal sleeper and paradoxical insomnia groups. On the other hand, wake index and WASO was higher among psychophysiological insomnia compared to the other groups. This condition supports the state of "insomnia without objective findings" about paradox insomnia as unlike subjective PSQI findings, all important objective sleep characteristics in this group was similar to normal sleepers.[46]

There were no significant differences in the levels of DHEA, SHBG, testosterone, estradiol, progesterone, 17-OH, FSH, and LH between two insomnia subgroups (paradox and psychophysiological) and normal sleepers. To the best of our knowledge, there is no previous study to investigate sex steroids in insomnia population. Similar to our

Table 3: Biochemical parameters among studied groups

	Normal (*n*=17) Male (*n*=13) Female (*n*=4)	Paradoxical insomnia (*n*=19) Male (*n*=6) Female (*n*=13)	Psychophysiological insomnia (*n*=17) Male (*n*=9) Female (*n*=8)	*P*
DHEA†				
Total	2.19 (0.63)[a]	1.92 (0.68)[a]	2.02 (0.65)[a]	0.67
Male	2.22 (0.70)[a]	2.01 (0.76)[a]	2.43 (0.47)[a]	0.48
Female	2.10 (0.38)[a]	1.87 (0.66)[a]	1.55 (0.48)[a]	0.26
SHBG				
Total	44.21 (24.48)[a]	44.69 (26.79)[a]	38.96 (22.13)[a]	0.86
Male	40.81 (18.68)[a]	28.34 (10.25)[a]	32.23 (17.75)[a]	0.28
Female	55.21 (39.86)[a]	52.23 (28.94)[a]	46.52 (25.20)[a]	0.86
Testosterone				
Total	2.89 (2.18)[a]	1.82 (2.38)[a]	1.97 (2.07)[a]	0.39
Male	3.68 (1.84)[a]	4.64 (1.99)[a]	3.51 (1.67)[a]	0.47
Female	0.29 (0.59)[a]	0.52 (1.03)[a]	0.23 (0.20)[a]	0.69
Estradiol				
Total	21.29 (31.80)[a]	42.57 (69.55)[a]	30.35 (43.20)[a]	0.57
Male	13.21 (6.41)[a]	9.93 (13.90)[a]	9.02 (5.50)[a]	0.47
Female	47.53 (63.47)[a]	57.63 (79.98)[a]	54.33 (54.67)[a]	0.96
Progesterone				
Total	0.407 (0.400)[a]	0.434 (0.316)[a]	0.489 (0.599)[a]	0.97
Male	0.46 (0.43)[a]	0.56 (0.34)[a]	0.34 (0.25)[a]	0.53
Female	0.21 (0.17)[a]	0.37 (0.29)[a]	0.65 (0.82)[a]	0.33
17-OH				
Total	1.805 (0.751)[a]	1.469 (0.430)[a]	1.734 (0.592)[a]	0.21
Male	1.75 (0.80)[a]	1.71 (0.46)[a]	1.67 (0.57)[a]	0.96
Female	1.98 (0.59)[a]	1.35 (0.37)[a]	1.80 (0.63)[a]	0.05
FSH				
Total	11.87 (14.11)[a]	18.54 (20.16)[a]	14.31 (17.38)[a]	0.72
Male	9.85 (5.37)[a]	13.18 (5.01)[a]	11.96 (7.16)[a]	0.48
Female	18.41 (29.51)[a]	21.00 (24.04)[a]	16.94 (24.82)[a]	0.93
LH				
Total	7.77 (6.91)[a]	6.07 (5.37)[a]	7.05 (10.18)[a]	0.84
Male	5.96 (5.52)[a]	2.63 (2.04)	7.55 (10.51)[a]	0.43
Female	13.75 (8.37)[a]	7.64 (5.73)[a]	8.48 (10.47)	0.31

†Mean (SD). Statistical analysis for the equality of the mean values among three groups was evaluated using ANCOVA adjusted by sex, age, and BMI ($P<0.05$). Means with the same superscript letters within a row were not significantly different ($P>0.05$).
DHEA-S – Dehydroepiandrosterone sulfate; SHBG – Sex hormone-binding globulin; 17-OH – 17α-Hydroxyprogesterone; FSH – Follicle stimulating hormone; LH – Luteinizing hormone; SD – Standard deviation; BMI – Body mass index

Table 4: Multiple analysis of polysomnography variables associated with hypothalamus–pituitary–gonadal hormones using the linear regression model adjusted by sex, age, and body mass index

Hormones	PSG variables	Coefficient	95% CI		*P*
			Lower	Upper	
SHBG	Diastolic blood pressure	1.37	0.12	2.61	0.03
Testosterone	Maximum pulse transit time	0.31	0.01	0.05	<0.01
FSH	Wake index	1.30	0.52	2.09	<0.01
	Diastolic blood pressure	1.29	0.42	2.15	<0.01
LH	Wake index	0.54	0.23	0.85	<0.01
	Diastolic blood pressure	0.34	0.001	0.68	0.04

CI – Confidence interval; SHBG – Sex hormone-binding globulin; FSH – Follicle stimulating hormone; LH – Luteinizing hormone

results, Auyeung and his colleagues found that there is no association between subjective insomnia complaint and early morning testosterone in >65–year-old men.[48] Previous studies on sleep fluctuations during menstrual cycle and menopausal status in women have revealed that the levels of sex hormones are related to sleep.[17,18] We could not find any report that directly investigated the sex hormones among people with insomnia diagnosis.

We did not observe any significant differences in the level of biochemical parameters between normal sleepers and two subtypes of insomnia. This finding may useful for modifying some maladaptive cognition in patients with insomnia tend to exaggerate the disorder. As they believe that there must be something really wrong prevent them going to sleep. According to previous studies on insomnia, sleeplessness negatively affects aspects of physical and mental health; however, our results did not support this idea. Our findings indicated that there is no significant association between the levels of sex hormones and the occurrence of all subtypes of insomnia. This result could be used for reassurance about the consequence of insomnia and modifying maladaptive cognition among insomniac patients.

In another part of our results, testosterone was positively related to maximum PTT. Some studies considered PTT as a sufficient measurement for stress.[32,49] Hence, the association between maximum PTT and serum testosterone might be related to ANS activity. Both animal and human studies confirmed the increase of serum testosterone level in response to stress through subsequent activation of HPA axis.[50,51] Studies indicated that EEG arousal index is not sufficient to reveal subcortical arousal events responsible for modulating respiratory and blood pressure conditions. Therefore, PTT index is more suitable for indicating subcortical arousal that responsible for vegetative and physiological consequences in the stress system.[52] Investigating subcortical arousal by PTT could lead to more precise investigation of insomnia. EEG monitoring of sleep reveals important information about neurophysiological bases of sleep disorders by recording surface electrophysiological activity of cortex. Therefore, EEG may fail to precisely reveal the role of deep brain structures such as reticular activating system as an important arousal modulator center. PTT as an index for subcortical arousality may provide physiological data to better understanding the subcortical involvement in insomnia.

Both LH and FSH were positively associated with wake index and diastolic blood pressure. These gonadotropins are secreted by gonadotropic cells in the anterior pituitary gland. The activity of gonadotropic cells is regulated by gonadotropin-releasing hormone (GnRH) neurons mainly located in preoptic area of hypothalamus. Interaction of sleep with preoptic area of hypothalamus has been reported, so far.[53] Sleep fragmentation that revealed by wake index may influence the preoptic area which consequently leads to higher activity of gonadotropic cells and increasing of GnRH. Previous studies conducted on puberty participants indicated that LH pulses occur most frequently during slow-wave sleep and less frequently during periods of wakefulness after sleep onset,[54] but the issue has not been considered in adults. Our results revealed that the wake index was positively associated with elevated serum level of LH and FSH. Moreover, diastolic blood pressure positively affects the level of gonadotropins. This finding may indicate the role of the stress system on activating the GnRH neurons in preoptic area.

Conclusion

The subjective and objective sleep architectures indicated sleep state misperception condition in paradoxical insomnia. There were no significant differences in all biochemical analysis between two insomnia subgroups (paradox and psychophysiological) and normal sleepers. Testosterone was positively related to maximum PTT. Moreover, both LH and FSH were positively associated with wake index and diastolic blood pressure. Doing further experimental studies on larger populations are also suggested.

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Conflicts of interest

There are no conflicts of interest.

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